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Ethylene and Senescence in Petals of *Tradescantia*[1]

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ABSTRACT

Flowers of *Tradescantia* (clone O2) which are ephemeral, produce ethylene during senescence with the maximum rates occurring during the initial period of fading. Senescing isolated petals produce ethylene in a similar manner, exhibit a loss of membrane semipermeability, and exogenous ethylene hastens the onset as well as the subsequent rate of this loss. The aminoethoxy analog of 0.1 millimolar rhizobitoxine completely inhibits ethylene production by isolated petals but only partially the loss of membrane semipermeability. Isolated petals acquire a sensitivity to ethylene as they mature, becoming fully sensitive on the day of anthesis.

Ethylene has been shown to be involved in the regulation of senescence in a variety of plant organs including fruits and flowers (1, 2, 7, 13-15). However, its mode of action in this process is not understood. Hanson and Kende (6) have shown that ethylene enhances loss of membrane semipermeability in mature petal tissue of *Ipomoea tricolor*. These results are consistent with the hypothesis that the tonoplast is the first membrane to be affected in the course of ethylene action (6, 8).

Flowers of *Tradescantia* are ephemeral and contain delphinidin (12), an anthocyanin pigment which is localized in the vacuole. Preliminary experiments indicated that isolated senescing petals lose this pigment to a bathing medium and that exogenous ethylene hastens the onset and the rate of senescence. Therefore, petals of *Tradescantia* should be a very suitable material for studying the action of ethylene on the integrity of the tonoplast and the consequences of tonoplast deterioration on the process of senescence itself.

MATERIALS AND METHODS

Plant Material. Cloned plants of a hybrid *Tradescantia* (O2 clone; putative parents *T. occidentalis* × *T. ohiensis*; obtained from L. Mericle, Dept. of Botany and Plant Pathology, M.S.U.) were planted in plastic pots in a 1:1:1 (v/v) mixture of potting soil, sand, and Perlite. The plants were watered twice daily and maintained under a daily regime of 16-hr light at 24 C and 8-hr dark at 20 C. The light intensity at plant height was 4 to 6 × 10^4 ergs cm^{-2} sec^{-1}, and the RH was maintained between 60 and 70%. Throughout this paper the following terminology will be used when referring to the plant material: day −2: flowers or flower parts isolated 2 days prior to flower opening; day −1: flowers or flower parts isolated 1 day prior to flower opening; and day 0: flowers or flower parts isolated on the day of opening.

Experiments with Intact Flowers. For the determination of ethylene synthesis in whole *Tradescantia* flowers, single flowers were excised from the plants early in the morning of day 0, and placed with their cut stems into nitrocellulose (7 × 15 mm) tubes containing distilled H_2O. Each tube was inserted into a perforated foam stopper which was placed into a 50-ml plastic syringe. The plunger of the syringe was adjusted to give an air space of 30 ml, and the syringe was sealed with a serum vial cap. The progress of flower fading was viewed through the plastic walls of the sealed syringes. In order to study the effect of an exposure of flowers to ethylene, batches of flowers were gassed simultaneously with 10 μl/l ethylene for 90 min in a 50-ml stoppered plastic syringe. Upon completion of this treatment, the flowers were removed and placed into individual 50-ml syringes as described above. The length of time from sealing this syringe until the flowers had completely closed was noted.

Ethylene Production by Isolated Floral Tissue. Flowers were excised from the plant early on day 0 and were dissected into sepals, petals, and the remaining organs (*i.e.* stamens, gynoecium, and receptacle). The respective parts from three flowers were placed into a 25-ml Erlenmyer flask containing 5 ml of 1% agar as support. The flasks were sealed, and ethylene determinations were made throughout the day.

Isolated Petals. Petals were isolated either from buds on day −1 or from flowers on day 0. The petals were floated on 5 ml of glass-distilled H_2O or solutions of the aminoethoxy analog of rhizobitoxine in 50-ml Erlenmeyer flasks with side arms; each flask was sealed with a serum vial cap and fitted with a conical cuvette attached with a 2.5-cm rubber tubing to the side arm. This assembly allowed continuous measurement of both ethylene concentration in the headspace and the absorbance of the bathing medium.

Determination of Pigment Efflux and Ethylene Production. Anthocyanin efflux was monitored in the sealed system by tipping the flask such that a portion of the bathing medium was introduced into the conical cuvette, thereby allowing for the measurement of the absorbance of the bathing medium at 575 nm, using a Coleman Junior Colorimeter (Coleman Instruments, Oak Brook, Ill.). Ethylene production was measured by withdrawing a 1-ml gas sample from the headspace in the incubation flask and injecting it into a gas chromatograph as described previously in studies with *Ipomoea* flower tissue (9). Each sample removed was replaced with 1 ml of ethylene-free air.

Electrolyte Leakage. Petals were isolated on the appropriate day early in the morning and were floated on glass-distilled H_2O for 1 hr. They were then placed on 8-ml glass-distilled H_2O in 50-ml flasks; the flasks were sealed with a serum vial cap. Ethylene was injected into half of the flasks to give a final concentration of 10 μl/l, and at the appropriate times 5 ml of bathing solution was removed and the conductance measured with a Markson Electro Mark Analyzer (Markson Science, Del Mar, Calif.). These 5 ml of solution were returned to the flask, the flask was resealed, and fresh air or ethylene (final concentration 10 μl/l) was reintroduced.

Effects of Ethylene on Endogenous Ethylene Synthesis. Petals of different ages were excised early in the morning and divided into two groups. Those to be exposed to ethylene were placed on water-soaked cotton inside a 30-ml test tube and the tube was sealed with a serum vial cap. Following the ethylene treatment, the petals were allowed to stand 5 to 10 min in laboratory air; after this both ethylene-pretreated and control petals were placed

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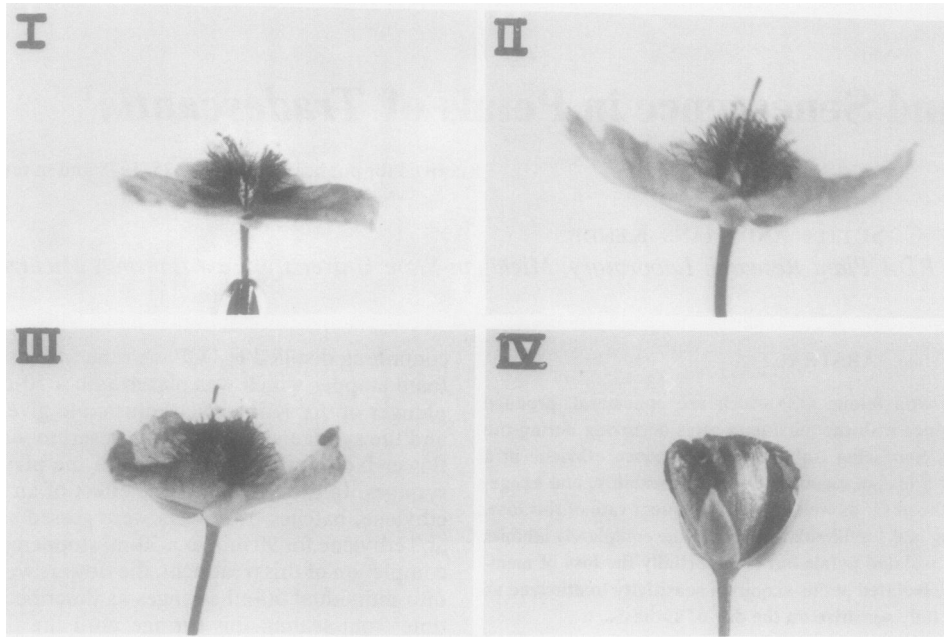

FIG. 1. Developmental (morphological) changes during senescence of *Tradescantia* flowers. Stage I: fully open; stages II and III: initiation and progression of fading; stage IV: fully faded flower.

into 25-ml flasks as described in the experiments on the production of ethylene by isolated floral tissues.

All experiments were repeated no less than four times, all giving comparable results.

RESULTS

Figure 1 shows the progress of floral senescence in intact *Tradescantia* flowers. The buds open early in the morning, becoming fully open by 10:00 hr (stage I). By 16:00 hr the petals begin to show signs of wilting beginning at the distal margins and proceeding basipetally (stages II and III). By 22:00 hr the flowers have closed irreversibly (stage IV), and the petals become translucent because of leakage of cell sap into the intercellular spaces.

When fully open flowers were excised from the plant and placed within a sealed 50-ml syringe, the flowers remained open for 5.25 (± 0.9) hr. If similar flowers were pretreated for 90 min with a 10 μl/l atmosphere of ethylene prior to sealing, they remained open for only 3.75 (± 0.5) hr. Figure 2 shows the relationship between morphology and the rate of ethylene production of flowers which had been kept on the plant until the rate of ethylene evolution was measured. There was an initial increase in the rate of ethylene production occurring slightly before curling of the petals. The rate of ethylene production remained high throughout senescence and fell off as the flowers became fully closed. From Figure 3 it is evident that all floral tissues produced ethylene with the major part originating from the reproductive organs (>70%). There was a substantial (20%) contribution by the isolated petals as well. Furthermore, both the reproductive organs and the petals produced ethylene throughout the day in a manner analogous to the production by the intact flower. However, the onset of ethylene production in the different organs was not synchronous. Ethylene could be detected in reproductive organs and receptacle tissue by 10:20 hr, while the initial increase in production by the petals commenced 3 hr later. These results show that there are several sources of ethylene production in the intact flower; however, signs of fading can be seen only in the petals. For this reason we decided to investigate the relationship between ethylene production and senescence in isolated petals, organs which not only produce ethylene but also respond to it visibly.

While isolated petals produced ethylene during the course of senescence, they did not curl like petals in intact flowers. They did, however, become translucent as the cell sap diffused into the intercellular spaces, and they subsequently lost their pigment to the bathing solution.

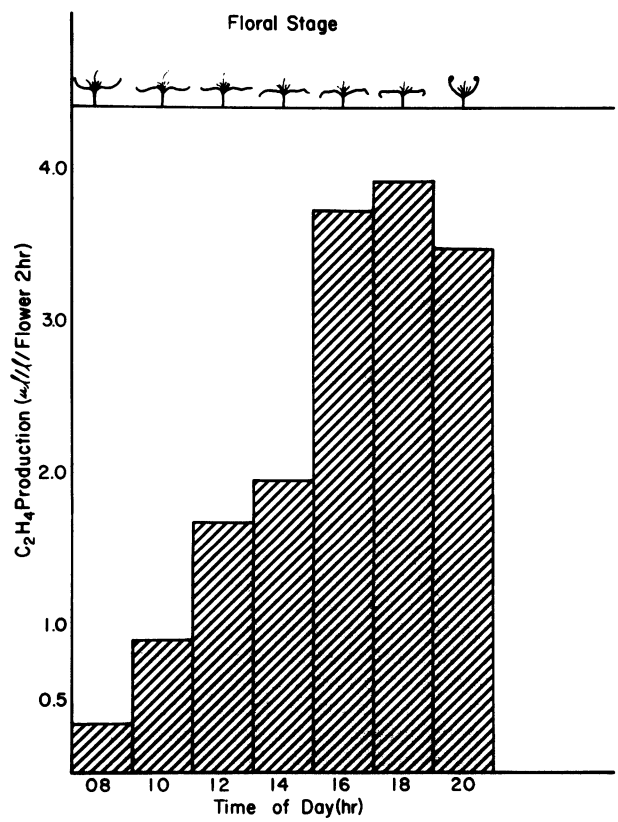

FIG. 2. Comparison of rate of ethylene production and morphology of *Tradescantia* flowers on day 0. Flowers were excised from the plant throughout the day and were placed inside a stoppered plastic syringe for 2 hr in order to determine the rate of ethylene production.

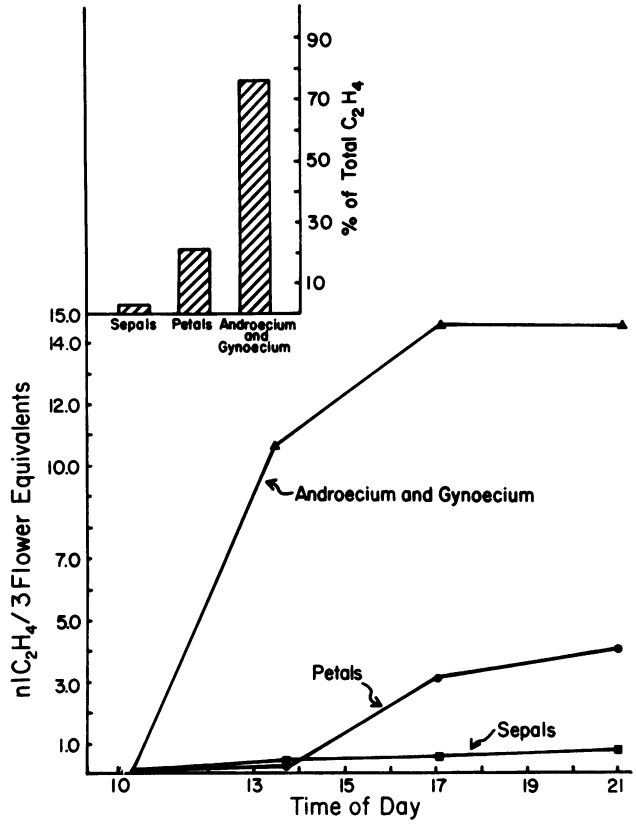

FIG. 3. Time course of ethylene production by isolated parts of *Tradescantia* flower. Flowers were dissected at 08:15 on day 0 and allowed to stand in a humid chamber until 10:00. The respective organs of three flowers were then placed into a 25-ml stoppered flask. Inset indicates per cent production of ethylene by each group of isolated organs.

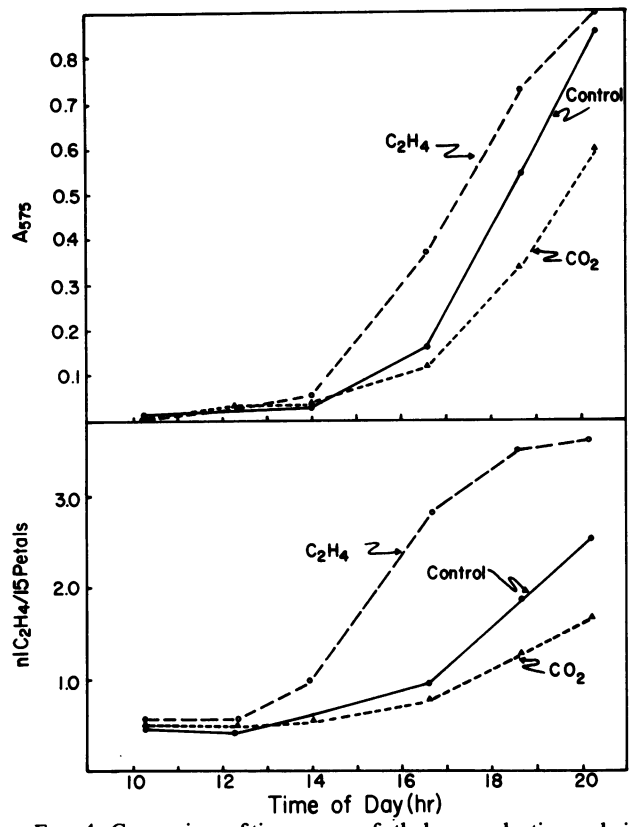

FIG. 4. Comparison of time course of ethylene production and pigment efflux in isolated *Tradescantia* petals on day 0. One group of petals was pretreated with 10 μl/l of ethylene from 09:00 to 10:00 and subsequently transferred to the experimental chamber; one group was placed into a chamber which was maintained at 4% (v/v) CO_2 throughout the experiment. Control petals received no ethylene or CO_2 treatment.

Figure 4 shows the relationship between ethylene evolution and pigment efflux in petals isolated on day 0. Both processes started simultaneously between 14:00 and 16:00 hr. In petals pretreated for 60 min with 10 μl/l ethylene, both pigment efflux and endogenous ethylene production started at least 2 hr earlier than in the control petals. Continuous exposure of isolated petals to 4% (v/v) CO_2 resulted in a retardation of both pigment efflux and ethylene production.

If petals were isolated from the bud on day −1 and kept overnight they senesced on day 0 in a manner similar to petals isolated on day 0 (Fig. 5). This fact permits feeding experiments such as those depicted in Figure 5.

When petals were isolated on day −1 and kept overnight on a 0.1 mM solution of the aminoethoxy analog of rhizobitoxine they exhibited no ethylene production. Figure 5 shows that although there was some ethylene present initially, the toxin-treated petals produced no detectable amounts of ethylene throughout the experiment. The petals still lost pigment, the efflux beginning simultaneously with that in control petals not treated with the toxin. However, the total amount of pigment loss throughout the day was considerably less than in control petals. A 60-min pretreatment with 10 μl/l ethylene restored the pigment efflux in toxin-treated petals to the control level, but ethylene production remained inhibited.

These experiments indicate that factors other than ethylene are also involved in petal senescence. To gain further insight into this, the effect of ethylene on immature petals was determined. Figure 6 illustrates the effect of ethylene on electrolyte leakage from petals which were isolated on day −2, day −1, and day 0. Petals isolated on day −2 showed a constant rate of efflux of electrolytes throughout the day, and continuous exposure of the petals to 10

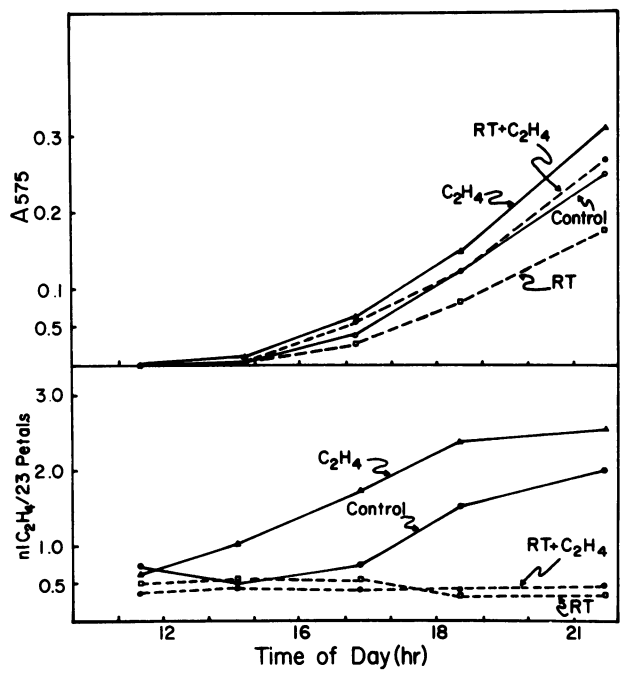

FIG. 5. Comparison of time course of ethylene production and pigment efflux in *Tradescantia* petals on day 0. Petals were isolated on the evening of day −1 and maintained on either distilled H_2O or a solution of 10^{-4} M aminoetheoxy analog of rhizobitoxine. (- - -): values for toxin-treated petals. One group of both toxin-treated and control petals were pretreated on the morning of day 0 for 60 min with 10 μl/l ethylene prior to being sealed in the experimental chamber.

μl/l ethylene had no effect on this process. Petals isolated on day −1 also exhibited a constant rate of electrolyte efflux through the day, the rate not differing greatly from those isolated on day −2. However, continuous exposure of these petals to 10 μl/l ethylene on day −1 caused an enhanced rate of electrolyte efflux. Although the petals were fully pigmented, there was no anthocyanin efflux from control and ethylene-treated petals on day −1. In petals isolated on day 0, applied ethylene hastened the leakage of electrolytes by 2 hr (note difference in scale). Also in control petals on day 0 there was a spontaneous increase in the rate of electrolyte leakage around 15:30, indicating the onset of endogenous senescence.

Figure 7 shows that a 90-min pretreatment with ethylene had no effect on subsequent ethylene production in petals isolated on day −1 or day −2. In fact, the ethylene pretreatment appeared to depress the rate of subsequent ethylene production in these petals. On day 0, ethylene production in control petals increased spontaneously around 15:00 hr. Pretreatment with ethylene shifted the onset of ethylene evolution to 13:00 hr.

DISCUSSION

Our results show that ethylene is a regulator of both flower fading and petal senescence in *Tradescantia*. This is concluded from the fact that exogenous ethylene accelerates the rate of both processes, and that the endogenous production of ethylene sharply

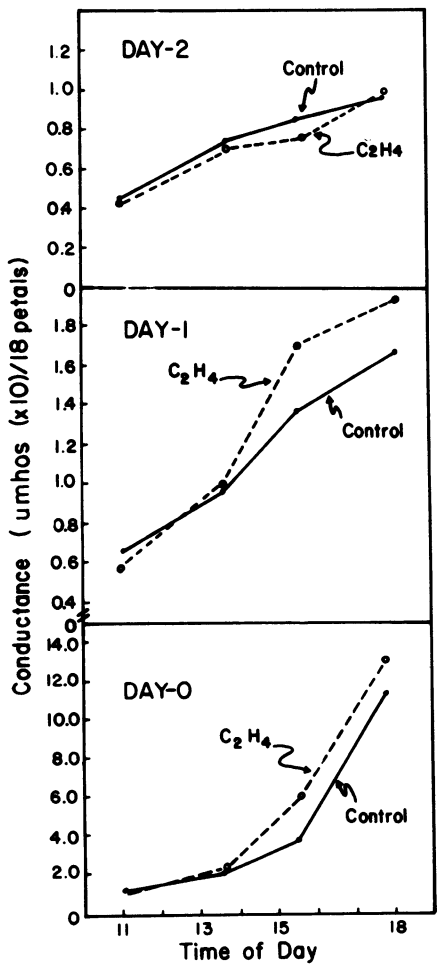

FIG. 6. Effect of 10 μl/l ethylene on electrolyte leakage from isolated *Tradescantia* petals during days −2, −1, and 0. Eighteen petals were floated on 8 ml of glass-distilled, deionized H_2O in a sealed 50-ml flask. (- - -): values for ethylene-treated petals.

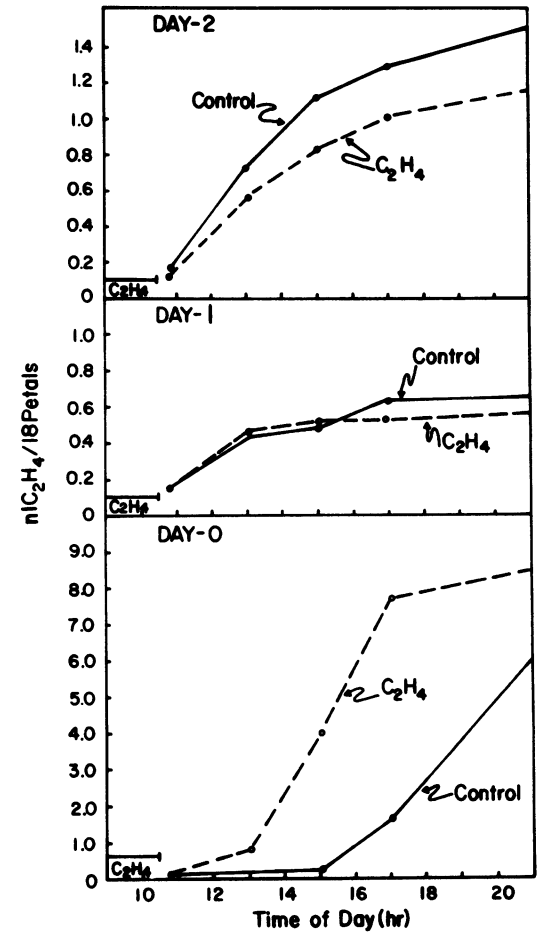

FIG. 7. Effect of 90-min, 10 μl/l ethylene pretreatment on subsequent rate of ethylene production in isolated *Tradescantia* petals on days −2, −1, and 0. Eighteen petals were placed into a 25-ml flask containing 5 ml of 1% agar. The pretreatment with ethylene was performed in a separate chamber from 09:00 to 10:30.

increases in both flowers and petals as they deteriorate. The extent to which endogenously produced ethylene is involved in the regulation of petal aging is most clearly seen in Figure 5. The rate of anthocyanin efflux, which serves as an indicator of senescence, is retarded in petals which have been treated with the aminoethoxy analog of rhizobitoxine, and a 60-min pretreatment with 10 μl/l ethylene is sufficient to restore the rate of pigment efflux from toxin-treated petals to the control rate.

Figure 3 shows that ethylene is produced by all floral tissues with the major contribution originating from the reproductive tissues. These data are consistent with similar observations made with cotton and carnation flowers (13, 15). The role of pollination in initiating ethylene production in flowers is well documented (1, 3, 5), and the substantial ethylene production by the reproductive tissues of *Tradescantia* flowers may well be another instance of such an interaction.

The role of ethylene in senescence has been interpreted in two, apparently conflicting ways. Some authors consider ethylene as the actual trigger of senescence (16); others feel that metabolic processes preceding ethylene synthesis lead to the onset of senescence, and that ethylene regulates the rate of the terminal deteriorative changes (9, 10). The results of Figure 5 tend to support the second hypothesis. Although there is no detectable ethylene production in the presence of the rhizobitoxine analog, the initiation of pigment leakage is not delayed even though the rate of efflux is reduced, indicating that factors other than ethylene may determine the initiation of senescence in isolated petals of *Tradescantia*.

Further support for the second hypothesis can also be derived from Figures 6 and 7. If ethylene is the trigger initiating senescence in *Tradescantia* petals, application of ethylene to immature petals which normally produce only small amounts of ethylene on day −2 and day −1 should induce senescence in these tissues as well. Our data show that petals isolated on day −2 are completely insensitive to exogenous ethylene. These petals exhibit no ethylene-enhanced electrolyte leakage nor ethylene-induced ethylene synthesis. One day prior to opening, exogenous ethylene does stimulate electrolyte leakage but has no effect on the rate of subsequent ethylene production. However, on the day of opening, exogenous ethylene accelerates the rates of both processes. As petals mature, they acquire a sensitivity to exogenous ethylene. The gradual acquisition of ethylene sensitivity has also been observed in flower tissue of *Ipomoea tricolor* (9) and in fruit tissue (2).

Results of other experiments concerning the effects of exogenous ethylene applied to petals on day −1 and day 0 point to further differences in the response of mature and immature tissues to the gas. Figures 4 and 6 demonstrate that exogenous ethylene stimulates both anthocyanin and electrolyte leakage from petals on day 0. When measured simultaneously, the rates of efflux of both were found to parallel each other throughout the day (not shown), indicating that ethylene increases membrane permeability in an unspecific fashion. However, exogenous ethylene causes no anthocyanin efflux in petals on day −1, even though they are fully pigmented. Therefore, the effect of ethylene in causing loss of cellular compartmentation is selective in these petals, and components involved in the "autocatalytic" synthesis of ethylene in petals on day 0 may remain sequestered in petals on day −1.

Because petals of *Tradescantia* are heavily pigmented, the effect of ethylene on membrane permeability can be measured easily. Furthermore, since anthocyanins are localized within the vacuole of all cells, the action of ethylene in enhancing the rate of efflux of this pigment can be interpreted as an effect of the gas on the integrity of the tonoplast. In other studies, utilizing solely electrolytes or radioactive tracers as markers of efflux, the effect of ethylene on the tonoplast could only be inferred indirectly (6). While a definite effect of the gas on the tonoplast has been shown in our present studies, a similar effect of ethylene on the permeability of the plasmalemma cannot be ruled out. It is impossible to say whether the permeability of the plasmalemma would have to increase to permit efflux of anthocyanin from the cell, once the pigment has been released from the vacuole into the cytoplasm.

The question that arises now concerns the physiological significance of the loss of tonoplast integrity. Matile (11) and others, using vacuole preparations from yeast and meristematic tissues, have provided evidence for the vacuolar localization of many hydrolases. Based on these data as well as numerous other observations, Matile has proposed the vacuole as the plant's equivalent of the lysosomal compartment of animal cells. If this hypothesis also holds true for vacuoles of mature plant tissues, the loss of tonoplast integrity would allow the mixing of heretofore sequestered vacuolar hydrolases with their cytoplasmic substrates. Loss of compartmentation between the vacuole and the cytoplasm may, in addition to causing eventual autolysis of the cell, also be the reason for "autocatalytic" ethylene production (6).

Recent evidence (4) using vacuoles derived from protoplasts of *Hippeastrum* petals does not support Matile's hypothesis. The majority of the hydrolytic activities in these petal protoplasts was found to be localized in the cytoplasm. Nevertheless, these authors envision another role of vacuolar contents in regulating cytoplasmic breakdown. In this scheme the release of ions (notably H^+) occurring as a result of increased tonoplast permeability would stimulate the activity of cytoplasmic acid hydrolases which would lead to the increased rate of macromolecular breakdown.

We have shown that *endogenous* ethylene is a regulator of senescence in *Tradescantia* flowers. It appears to play a decisive role in determining the rate of loss of compartmentation in *Tradescantia* petals, and this loss of compartmentation could play a central role in bringing about the deteriorative changes in the petals which occur during senescence.

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